
Leelaveni, A., Swarnalata Patnaik* and A. K. Panigrahi,
Environmental Toxicology Laboratory, Department of Botany, Berhampur University, BERHAMPUR-760 007, Odisha, India.
*M.M.Womens College, Berhampur-760007, Odisha, India

Highlights:
- The extract of the red mud pond contaminates the surrounding environmental segments, and crop fields nearby. The extract leaking from the red mud pond is highly toxic.
- The extract drastically affected the biomolecular content (DNA, RNA, Protein & FAA content of the control and RMWE exposed leaf and root of green gram plants at different exposure periods.
- All the 4 macromolecules decreased with the increase in exposure period and increase in alumina residue in the tissues showing a positive correlation.
- The percent decrease in biomolecular parameters increased with the increase in exposure period showing a positive correlation.
- Significant residual accumulation of alumina was observed in shoot and root of the RMWE exposed green gram plants in pot culture.

Abstract
The main objective of the study was to study the impact red mud waste extract on a crop plant. The red mud waste extract (RMWE) prepared in the laboratory is equivalent to the extract chemicals leaking from the red mud pond. The DNA, RNA, protein and FAA content significantly decreased in lechate exposed green gram seedlings in petriplate culture compared to control values indicating significant inhibition. Little insignificant increment in FAA and RNA in lechate exposed seedlings did not indicate any impact except the fact that increases in FAA in exposed seedlings might be a case of proteolysis in the exposed seedlings. Significant decrease in the DNA, RNA, protein and FAA content in lechate exposed green gram seedlings in pot culture compared to control values were noted. These biomolecular depletion no doubt indicate significant inhibition caused by the toxicant (RMWE). Little insignificant increment in FAA and RNA in lechate exposed seedlings did not indicate any impact except the fact that increases in FAA in exposed seedlings might be a case of proteolysis in the exposed seedlings as stated earlier. Significant decrease in DNA content and depletion in protein content was probably due to cell death and proteolysis caused by the toxicant. The leaf and root showed accumulation of alumina and maximum deposition was noted in the roots compared to leaf. No residual alumina accumulation was observed in the seeds of green gram plants after prolonged exposure to lechate waste, hence this crop can be recommended for cultivation in the contaminated site instead of rice cultivation.

Keywords: Red Mud waste, Alumina, RMW Extract, crop plant, DNA, RNA, Protein, FAA.
Introduction

Alumina industries pollute air, water and land mass to the maximum extent possible. Red mud waste is generally dumped near a safe place and generally kept away from water bodies and human habitation. Red mud pond located at Damonjodi is surrounded by natural hills from all sides and only at one side a dyke is constructed to protect the red mud waste. The red mud waste contains many heavy metals and metalloids. The pH of red mud waste is highly alkaline. The red mud waste contains toxic chemicals like Cd, Ar, Cu, Pb, Hg, Al, Co, Ni, Zn, Fe etc. Several heavy metal ions are reported to cause preoccupation of membrane lipids (plasma and chloroplast membrane). To mitigate the environmental issues of heavy metal contamination and food security, agricultural product safety, drinking water safety and protection of all economically important plants and animals different strategies are planned either by using physico-chemical treatment technology, chemical washing, electro-remediation, bioremediation, phyto-remediation etc were tested, planned and executed for a safer environment and a safer living (Shumakar and Begonia, 2005). These processes either remove the heavy metals from the environment or make heavy metal inactive or not available for absorption. Stabilizing the heavy metals is cost effective, a best option for many to reduce toxicity, but we do not agree with this hypothesis. Xu et al, (2021) rightly pointed out the urgent necessity of some new technology to remediate cadmium polluted farmland. NALCO (National Aluminium Co. Limited at Damonjodi, Koraput, Odisha) is the leading industry of World standard for producing Alumina and India became self sufficient in Aluminium production and requirement (Patnaik et al., 2017,2018,2022; Panda et al., 2017, 2018). The industry during processing of the raw materials uses many chemicals, alters physical conditions at different steps to purify the product. In the whole sequences of getting pure alumina, the industry releases many of the chemicals as gases or liquids or solids at different stages of manufacture. The gases are generally released to air through chimneys which causes air pollution. To save the local people from air pollution the height of the chimney is raised to a great height, so that the released polluted air moves to distances. The liquid waste is very thick (red mud=RM) called as slurry is discharged into a stocking area (RM Pond) for air drying the red mud waste. The red mud waste is stored in the red mud pond which is surrounded by natural hills from all sides and a small link is left out which is closed by earthen dam in the form of a dam. Earlier no body probably thought of leakage and leaching. The pond is not plastic lined or cemented. Continuous leaching takes place from red mud pond and the surrounding land mass is contaminated by the leached chemicals. After many years, now it was observed that small line steams are oozing from the red mud pond and flows downwards and joins small streams and finally these streams join and reach to a small river contaminating the aquatic system. It was also observed that the extract flow towards crop fields located nearby. These extract accumulate in the crop fields and keep the field wet. Cultivation was earlier easy due to the availability of moisture in the fields. The study of Patnaik et al., (2018, 22, 23) clearly indicated the impact of red mud waste extract on the seed biological parameters of green gram seeds in petriplate and pot culture. It was planned to further strengthen the information by conducting tests on growing plants in pot culture. This project aims to evaluate the eco-toxicological effects of the leached waste of the Red Mud Pond of Alumina industry / RMWE prepared on the growth and physiological activity of a green gram crop plant under laboratory controlled conditions.

Material & Methods:

Location of the industry: The mines and refinery complex of NALCO, Damonjodi is situated at Similiguda block, under Potangi tahasil in the district of Koraput, Odisha, India. The industry is located at latitude 18°-6'-18°-58’ towards North and longitude 82°.57’-83°.04’ East.

Toxicant: Red mud waste extract: The extract is prepared as explained by Patnaik et al., (2023) in the laboratory and used for the pot culture experiments.

Test system: Green gram seeds (Eng. Mung, Green gram; Odia: Muga)

Botanical name of the crop plant: Vigna radiata, (L.) Wilczek

Pure line uncontaminated seeds of green gram (Odia: Muga) were obtained from Pulses and Millet Research Station, OUAT, Ratanpur, Ganjam.

Pot culture experiments were conducted at sub-lethal concentrations and LC10 dose of the red mud waste extract under laboratory controlled conditions.

Total DNA was measured following the procedure as explained by Herbert et al., (1971) by diphenylamine reaction method and RNA by Orcinol reagent method of Volkin and Cohn (1954). The amount protein was estimated by the procedure of Lowry et al. (1951) & the protocol followed (Radha, 2004). FAA content was estimated by Ninhydrin method following the procedure of Lee & Takahasi (1966). The soil, red mud waste, red mud waste extract and plant samples were digested with acid digestion mixture and the digested samples were estimated for residual alumina by AA Spectrophotometer.
Results

Analysis of mud of red mud pond (Patnaik et al., 2022)

Analysis of red mud waste revealed that the waste is a mixture of the following chemicals expressed in percentages: Al₂O₃ = 19.11%; Fe₂O₃ = 56.88%; TiO₂ = 4.56%; SiO₂ = 5.97%; Na₂O = 3.86%; pH = 9.90; Return water pH = 13.6; NaOH = 30-35% and Na₂CO₃ = 65-70%. From the toxicity test, the lethal concentration values for green gram seeds deduced were LC₀ = 0.075 ml/liter (v/v); LC₀₅ = 0.2 ml/liter; LC₁₀₀ = 1.5 ml/liter; LC₅₀ = 3.0 ml/liter; LC₉₀ = 5.0 ml/liter and LC₁₀₀ = 5.6 ml/liter when exposed to extract (RMWE) of the red mud waste in pot culture (Patnaik et al., 2022).

Fig. 1 shows the changes in DNA content in control extract exposed green gram plants at different exposure periods and at different RMW extract concentrations. The DNA content of the green gram plant leaves ranged between 0.35±0.02 mg/g fresh weights to 0.38±0.05 mg/g fresh weight during the entire period of experimentation (60days- at 15d interval) in the control pot set. The DNA content of the extract exposed green gram plant leaves decreased from 0.38±0.05 mg/g fresh weight to 0.32±0.06 mg/g fresh weight at conc. A after 15days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.38±0.05 mg/g fresh weight to 0.24±0.08 mg/g fresh weight at conc. B after 15days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.38±0.05 mg/g fresh weight to 0.11±0.05 mg/g fresh weight at conc. C after 15days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.36±0.04 µg/g fresh weight to 0.27±0.02 mg/g fresh weight at conc. A after 30days of exposure.

The DNA content of the extract exposed green gram plant leaves decreased from 0.36±0.04 mg/g fresh weight to 0.21±0.03 mg/g fresh weight at conc. B after 30days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.36±0.04 mg/g fresh weight to 0.12±0.06 mg/g fresh weight at conc. C after 30days of exposure in pot culture (Fig.1). The DNA content of the extract exposed green gram plant leaves decreased from 0.35±0.02 mg/g fresh weight to 0.21±0.04 mg/g fresh weight at conc. A after 45days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.35±0.02 µg/g fresh weight to 0.19±0.07 µg/g fresh weight at conc. B after 45days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.35±0.02 mg/g fresh weight to 0.11±0.04 mg/g fresh weight at conc. C after 45days of exposure in pot culture. The DNA content of the extract exposed green gram plant leaves decreased from 0.37±0.04 mg/g fresh weight to 0.19±0.02 mg/g fresh weight at conc. A after 60days of exposure. The DNA content of the extract exposed green gram plant

Fig. 1: Changes in DNA content in the leaf and root of control and RMWE exposed green gram plants at different exposure periods.

Fig. 2: Percent decrease in DNA content in leaf and root of green gram lechate exposed plants in pot culture.

Fig. 3: Changes in RNA content in leaf and root of control and lechate exposed green gram plants at different exposure periods.

Fig. 4: Percent changes in RNA content in leaf and root of control and lechate exposed green gram plant seedlings at different exposure periods.
leaves decreased from 0.37±0.04mg/g fresh weight to 0.18±0.06mg/g fresh weight at conc. B after 60 days of exposure. The DNA content of the extract exposed green gram plant leaves decreased from 0.37±0.04mg/g fresh weight to 0.05±0.02mg/g fresh weight at conc. C after 60 days of exposure in pot culture. The DNA content of the green gram plant roots ranged between 0.4±0.05mg/g fresh weights to 0.42±0.04mg/g fresh weight during the entire period of experimentation in the control pot set. The DNA content of the extract exposed green gram plant leaves decreased from 0.4±0.05mg/g fresh weight to 0.07±0.05mg/g fresh weight at conc. C after 45 days of exposure in pot culture. The DNA content of the extract exposed green gram plant roots decreased from 0.4±0.05mg/g fresh weight to 0.16±0.09mg/g fresh weight at conc. A after 60 days of exposure. The DNA content decreased by 15.9%, 36.8% and 71.1% at conc. A, conc. B and conc. C after 15 days of exposure in the extract exposed leaves of green gram plants. The DNA content decreased by 25%, 41.7% and 66.7% at conc. A, conc. B and conc. C after 30 days of exposure in the extract exposed leaves of green gram plants. The DNA content decreased by 40%, 45.7% and 68.6% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed leaves of green gram plants. The DNA content decreased by 48.7%, 51.4% and 86.5% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed leaves of green gram plants. The DNA content decreased by 41.5%, 63.4% and 73.2% at conc. A, conc. B and conc. C after 30 days of exposure in the extract exposed roots of green gram plants. The DNA content decreased by 52.4%, 73.8% and 83.3% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed roots of green gram plants. The DNA content decreased by 60%, 80% and 92.5% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed roots of green gram plants (Fig. 2).

Fig.3 shows the changes in RNA content in control extract exposed green gram plants at different exposure periods and at different RMW extract concentrations. The RNA content of the green gram plant leaves ranged between 11.6±0.4µg/g fresh weights to 12.2±0.6µg/g fresh weight during the entire period of experimentation in the control pot set. The RNA content of the extract exposed green gram plant leaves increased from 11.6±0.4µg/g fresh weight to 12.4±0.6µg/g fresh weight at conc. A after 15 days of exposure. The RNA content of the extract exposed green gram plant leaves decreased from 11.6±0.4µg/g fresh weight to 6.1±0.5µg/g fresh weight at conc. C after 15 days of exposure (Fig. 3). The RNA content of the extract exposed green gram plant leaves decreased from 12.2±0.6µg/g fresh weight to 11.2±0.3µg/g fresh weight at conc. A after 30 days of exposure. The RNA content of the extract exposed green gram plant leaves decreased from 12.2±0.6µg/g fresh weight to 9.4±0.4µg/g fresh weight at conc. B after 30 days of exposure. The RNA content of the extract exposed green gram plant leaves decreased from 12.2±0.6µg/g fresh weight to 5.4±0.2µg/g fresh weight at conc. C after 30 days of exposure. The RNA content of the extract exposed green gram plant leaves decreased from 12.1±0.9µg/g fresh weight to 7.1±0.4µg/g fresh weight at conc. A after 60 days of exposure. The RNA content of the extract exposed green gram plant leaves decreased from 12.1±0.9µg/g fresh weight to 5.1±0.2µg/g fresh weight at conc. B after 60 days of exposure. The RNA content of the extract exposed green gram plant leaves decreased from 12.1±0.9µg/g fresh weight to 2.1±0.5µg/g fresh weight at conc. C after 60 days of exposure in pot culture (Fig. 3). The RNA content of the extract exposed green gram plant roots ranged between 13.1±0.6µg/g fresh weight to 13.8±0.4µg/g fresh weight during the entire period of experimentation in the control pot set. The RNA content of the extract exposed green gram plant roots decreased from 13.1±0.6µg/g fresh weight to 10.1±0.3µg/g fresh weight at conc. A after 15 days of exposure. The RNA content of the extract exposed green gram plant roots decreased from 13.1±0.6µg/g fresh weight to 9.2±0.4µg/g fresh weight at conc. B after 15 days of exposure. The RNA content increased by 6.9% at sub-lethal conc. A on 15th day of exposure in extract exposed green gram plants in pot culture. With the increase in extract concentration, the RNA content decreased by 21.6% and 47.4% at conc. A, conc. B and conc. C after 15 days of exposure in the extract exposed leaves of green gram plants. The RNA content decreased by 13.6%, 38.9% and 61.8% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed leaves of green gram plants. The RNA content decreased by 41.3%, 57.8% and 82.6% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed leaves of green gram plants. The RNA content decreased by 22.9%, 29.7% and 55.8% at conc. A, conc. B and conc. C after 15 days of exposure in the extract exposed roots of green gram plants. The RNA content decreased by 39.7%, 66.2% and 80.9% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed roots of green gram plants. The RNA content decreased by 47.8%, 75.4% and 93.5% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed roots of green gram plants (Fig. 4).

Fig.5 shows the changes in protein content in control extract exposed green gram plants at different exposure periods and at different RMW extract concentrations. The protein content of the green gram plant
leaves ranged between 20.6±1.4µg/g fresh weight to 24.1±2.8µg/g fresh weight during the entire period of experimentation in the control pot set. The protein content of the extract exposed green gram plant leaves decreased from 20.6±1.4µg/g fresh weight to 12.9±0.4µg/g fresh weight at conc. A after 15days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 20.6±1.4µg/g fresh weight to 7.4±0.9µg/g fresh weight at conc. C after 15days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 21.2±2.2µg/g fresh weight to 15.1±3.4µg/g fresh weight at conc. A after 30days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 22.3±3.4µg/g fresh weight to 13.1±1.8µg/g fresh weight at conc. A after 45days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 22.3±3.4µg/g fresh weight to 8.9±0.7µg/g fresh weight at conc. B after 45days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 22.3±3.4µg/g fresh weight to 4.8±0.3µg/g fresh weight at conc. C after 45days of exposure in pot culture. The protein content of the extract exposed green gram plant leaves decreased from 24.1±2.8µg/g fresh weight to 11.2±1.6µg/g fresh weight at conc. A after 60days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 24.1±2.8mg/g fresh weight to 2.2±0.3mg/g fresh weight at conc. C after 60days of exposure in pot culture. The protein content of the extract exposed green gram plant leaves decreased from 21.4±1.2mg/g fresh weight to 4.8±0.2mg/g fresh weight at conc. C after 15days of exposure (Fig.5). The protein content of the extract exposed green gram plant roots decreased from 22.3±2.3µg/g fresh weight to 8.4±0.9µg/g fresh weight at conc. B after 30days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 22.3±2.3µg/g fresh weight to 0.8±0.2µg/g fresh weight at conc. C after 30days of exposure in pot culture (Fig.5). The protein content of the extract exposed green gram plant leaves decreased from 24.5±3.1µg/g fresh weight to 11.2±1.4µg/g fresh weight at conc. A after 45days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 24.5±3.1µg/g fresh weight to 8.8±0.8µg/g fresh weight at conc. B after 45days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 24.5±3.1µg/g fresh weight to 2.4±0.3µg/g fresh weight at conc. C after 45days of exposure in pot culture (Fig.5).
The protein content of the extract exposed green gram plant roots decreased from 25.1±1.6 µg/g fresh weight to 9.6±0.4 µg/g fresh weight at conc. A after 60 days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 25.1±1.6 µg/g fresh weight to 4.5±1.1 µg/g fresh weight at conc. B after 60 days of exposure. The protein content of the extract exposed green gram plant leaves decreased from 14.2±1.4 µg/g fresh weight to 6.2±0.3 µg/g fresh weight during the entire period of experimentation in the control pot set. The FAA content of the extract exposed green gram plant leaves increased from 12.4±2.2 µg/g fresh weight to 16.1±1.4 µg/g fresh weight at conc. A after 15 days of exposure. The FAA content of the extract exposed green gram plant leaves increased from 11.2±1.4 µg/g fresh weight to 13.9±0.9 µg/g fresh weight at conc. B after 15 days of exposure. The FAA content of the extract exposed green gram plant leaves increased from 11.2±1.4 µg/g fresh weight to 2.8±0.6 µg/g fresh weight at conc. C after 60 days of exposure in the extract exposed roots of green gram plants. The FAA content decreased by 67.3%, 87.7% and 97.2% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed roots of green gram plants. The FAA content decreased by 39.9%, 59.2% and 86.1% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed leaves of green gram plants. The FAA content decreased by 58.4%, 69.8% and 95.5% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed roots of green gram plants. The FAA content decreased by 53.5%, 74.5% and 90.8% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed leaves of green gram plants in petriplate culture (Fig. 5).

Fig. 7 shows the changes in FAA content in control and RMW extract exposed green gram plants at different exposure periods and at different toxicant concentrations. The FAA content of the green gram plant leaves ranged between 11.2±1.4 µg/g fresh weights to 14.2±1.4 µg/g fresh weight during the entire period of experimentation in the control pot set. The FAA content of the extract exposed green gram plant leaves increased from 12.4±2.2 µg/g fresh weight to 16.1±1.4 µg/g fresh weight at conc. A after 15 days of exposure. The FAA content of the extract exposed green gram plant leaves increased from 13.3±1.8 µg/g fresh weight to 16.1±1.4 µg/g fresh weight at conc. A after 45 days of exposure. The FAA content of the extract exposed green gram plant leaves decreased from 14.2±2.2 µg/g fresh weight to 5.2±0.8 µg/g fresh weight at conc. C after 45 days of exposure. The FAA content of the extract exposed green gram plant leaves decreased from 14.2±1.4 µg/g fresh weight to 5.1±1.2 µg/g fresh weight at conc. B after 60 days of exposure. The FAA content of the extract exposed green gram plant leaves decreased from 14.2±2.2 µg/g fresh weight to 2.8±0.6 µg/g fresh weight at conc. C after 60 days of exposure in pot culture. The FAA content of the green gram plant roots ranged between 28.8%, 46.2% and 71.2% at conc. A, conc. B and conc. C after 30 days of exposure in the extract exposed leaves of green gram plants. The protein content decreased by 10.7%, 52.3% and 77.6% at conc. A, conc. B and conc. C after 15 days of exposure in the extract exposed roots of green gram plants. The protein content decreased by 39.9%, 59.2% and 86.1% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed roots of green gram plants. The protein content decreased by 58.4%, 69.8% and 95.5% at conc. A, conc. B and conc. C after 45 days of exposure in the extract exposed roots of green gram plants. The protein content decreased by 53.5%, 74.5% and 90.8% at conc. A, conc. B and conc. C after 60 days of exposure in the extract exposed roots of green gram plants (Fig. 6).
12.4±1.6µg/g fresh weight to 15.1±1.6µg/g fresh weight during the entire period of experimentation (60days- at 15d interval) in the control pot set. The FAA content of the extract exposed green gram plant roots increased from 12.4±1.6µg/g fresh weight to 14.2±2.8µg/g fresh weight at conc. A after 15days of exposure. The FAA content of the extract exposed green gram plant roots decreased from 12.4±1.6µg/g fresh weight to 12.4±1.4µg/g fresh weight at conc. B after 15days of exposure. The FAA content of the extract exposed green gram plant roots decreased from 12.4±1.6µg/g fresh weight to 5.7±1.3µg/g fresh weight at conc. C after 15days of exposure. The FAA content of the extract exposed green gram plant roots decreased from 15.1±1.6µg/g fresh weight to 10.9±2.2mg/g fresh weight at conc. A after 60days of exposure. The FAA content of the extract exposed green gram plant roots decreased from 15.1±1.6µg/g fresh weight to 9.2±1.2µg/g fresh weight at conc. B after 60days of exposure. The FAA content of the extract exposed green gram plant roots decreased from 15.1±1.6mg/g fresh weight to 1.2±0.2µmg/g fresh weight at conc. C after 60days of exposure in pot culture (Fig.7). The FAA content increased by 30.4% at sub-lethal conc. A on 15th day of exposure in extract exposed green gram plants in pot culture. With the increase in extract concentration, the FAA content increased by 24.1% and decreased by 39.3% at conc. B and conc. C after 15days of exposure in the extract exposed leaves of green gram plants. The FAA content increased by 22.6%, and then decreased by 4.8% and 50% at conc. A, conc. B and conc. C after 30days of exposure in the extract exposed leaves of green gram plants respectively. The FAA content initially increased by 21.1% and then decreased by 40.6% and 60.9% at conc. A, conc. B and conc. C after 45days of exposure in the extract exposed leaves of green gram plants. The FAA content increased by 22.5% at conc. A and then decreased by 64.1% and 80.3% at conc. B and conc. C after 60days of exposure in the extract exposed leaves of green gram plants (Fig. 8). The FAA content increased by 14.5% at conc. A and then the FAA content decreased by 1.6% and 54% at conc. B and conc. C after 15days of exposure in the extract exposed roots of green gram plants. The FAA content decreased by 12.3%, 18.8% and 76.8% at conc. A, conc. B and conc. C after 30days of exposure in the extract exposed roots of green gram plants. The FAA content decreased by 19.1%, 24.8% and 90.1% at conc. A, conc. B and conc. C after 45days of exposure in the extract exposed roots of green gram plants. The FAA content decreased by 27.8%, 39.1% and 92.1% at conc. A, conc. B and conc. C after 60days of exposure in the extract exposed roots of green gram plants (Fig. 8).

Fig. 9-10 shows residual accumulation of alumina in root and leaf of green gram plants grown in pot cultures at different periods of exposure and at different extract concentrations. No alumina was recorded in the control set green gram plant leaves. The leaves of extract exposed green gram plants accumulated 1.24±0.34µg /g dry weight; 2.42±0.38µg /g dry weight; 4.38±0.54µg /g dry weight and 5.26±0.66µg /g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 1.5mg/liter extract concentration. The residual accumulation of alumina increased in extract exposed green gram plant leaves with the increase in exposure period in pot culture. The leaves of extract exposed green gram plants accumulated 2.38±0.52µg/g dry weight; 3.65±0.24µg/g dry weight; 5.91±0.38µg/g dry weight and 8.45±1.12µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 3.0mg/liter extract concentration. The leaves of extract exposed green gram plants accumulated 2.71±0.46µg/g dry weight; 5.28±0.65µg/g dry weight; 7.34±0.46µg/g dry weight and 9.98±1.44 µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 5.0mg/liter extract concentration (Fig. 10). The root of extract exposed green gram plants accumulated 2.14±0.61µg /g dry weight; 2.95±0.46µg /g dry weight; 4.65±0.66µg /g dry weight and 5.69±0.71µg /g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 1.5mg/liter extract concentration. The residual accumulation of alumina increased in extract exposed green gram plant roots with the increase in exposure period in pot culture. The roots of extract exposed green gram plants accumulated 2.88±0.45µg/g dry weight; 4.12±0.52 µg/g dry weight; 6.88±0.32µg/g dry weight and 9.11±0.68µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 3.0mg/liter extract concentration. The roots of extract exposed green gram plants accumulated 3.26±0.52µg/g dry weight; 5.98±0.34µg /g dry weight; 7.28±0.69µg/g dry weight and 11.55±0.64µg/g dry weight after 15, 30, 45 and 60days of exposure respectively in pot culture at 5.0mg/liter extract concentration (Fig. 10). The leaf and root of control green gram plants did not show any residual accumulation of alumina indicating as standard control. The figure indicated clearly that the roots of the exposed green gram crop plants accumulated more amount of alumina compared to the leaves of the green gram crop plants in pot culture and the amount of alumina increased with the increase in exposure period and extract concentration. The ANOVA test indicated the existence of significant differences between rows and columns.
Discussion

Generally people of the area cultivate rice and rice is their staple food. These extract are light brown in color. It was observed that the rice crop absorbs these extract and the color of the leaf, root and leaf petiole slowly turn brown. This colour also passes into the grain/seed. The rice grain also looked brown. The rice once cooked, the cooked rice also turns light brown or light yellow and has a very bad smell. This rice grain was neither fit for consumption or for sale. Studies on heavy metal tolerance in plants indicated that root growth is particularly sensitive to heavy metals (Punz and Sieghardt, 1993). Copper and Cadmium in combination have affected adversely the germination, seedling length, and number of lateral roots in Solanum melongena (Neelima and Reddy, 2002). Reduction in root growth due to heavy metals has also been reported in wheat seedlings (Oncel et al., 2000). The number of leaves and branches, root and shoot length and biomass decreased as concentration of Cr increased in egg plant and tomato (Purohit et al., 2003) and in barley (Aery and Rana, 2003). The dry matter and yield of many higher plants such as pea, wheat, rape seed and maize have been reported to decrease under multiple heavy metal stress. Presence of Zn at higher concentrations retarded the growth and development of plants by interfering with certain important metabolic processes (Alia et al., 1995). The length of root and shoot were smaller for plants growing in Cadmium polluted soil. Significant reductions in length of Solanum melongena (Mehindirata et al., 1999) and plant height and fresh and dry matter in Brassica juncea (Singh and Tewari, 2003) at higher Cadmium concentration have also been reported. Mostly edible parts of plants are the major source of heavy metal intake for human through consumption, which have long-term detrimental effects on human health. Several heavy metals are considered toxic metals due to adverse human health effects, when taken in excess. Heavy metals pose hazards to human health because these are persistent in nature and have accumulation tendency in biological systems. Many authors indicated that nickel reduced the photosynthetic activity of nickel exposed plants (Mohanty et al., 1989; Sheoran et al., 1990; Krupa et al., 1993). Depletion of photosynthetic activity may result both due to the disturbance of photochemical and biochemical photosynthetic reduction and the damage of photosynthetic apparatus at all levels of organization (Mohanty et al., 1989) as reported in Cajanus cajan (Sheoran et al., 1990), Phaseolus vulgaris (Krupa et al., 1993) and Brassica oleracea (Molas, 2002). Disturbance and inhibition of carbohydrate transport from the place of photosynthesis result into the accumulation of starch under the influence of Ni in pine and birch (Kukkola and Huttunen, 1998) and rice (Moya and Ros, 1993). Net photosynthesis rate reduced significantly in mature leaves of Spinacea oleracea (Barua and Jana, 1986) and mung bean (Gadallah, 1995) due to heavy metal accumulation. Cadmium reduced leaf area, net photosynthesis rate and transpiration rate in corn and sunflower (Bazzaz et al., 1974 b), pea and sugar beet (Greger and Johansson., 1992). zinc and cadmium both inhibited photosynthetic CO2 fixation and Hill reaction activity of isolated spinach chloroplasts (Barua and Jana, 1986) and photosynthetic electron transport in isolated barley chloroplasts (Tripathi and Mohanty, 1980). In Zea mays, cadmium altered the photosynthesis and enzymes of photosynthetic sulphate and nitrate assimilation pathways (Ferretti et al., 1993). Mercury, Copper and zinc ions have changed the hydrolytic activity of chlorophyllase in rice leaves, with Hg increasing the activity maximally out of the three metals (Drazkiewicz, 1994). Heavy metal induced oxidative damage in senescing oat leaf cells (Luna et al., 1994), primary leaves of mung bean (Weckex and Clijsters, 1997) and in wheat leaves (Panda and Patra, 2000). The level of heavy metal translocated to chloroplast is estimated to be only about 1%, however, it varies from species to species. Kovacs-Bokor et al., (2018) reported significant effect of industrial sludge-soil mixture on wheat and white mustard by stimulating the germination of seeds and growth of plumule in exposed plants compared to standard control seed germination and plumule growth. The authors working on Niger on bauxite mining area indicated that the mining area chemicals have no effect or Niger plant can tolerate and sustain the wastes as evinced from high plant biomass, no change in pigment content. It was further supported by increase in some antioxidants in the exposed plants and in turn this supports tolerance to metals and its detoxification.

In the present study an attempt was made to understand the possibilities of reclamation of these heavy metal contaminated sites. Red mud is a highly alkaline dangerous toxic waste. Its toxicity decreases in acid soil. In some places red mud waste is used to reclaim cadmium polluted soils. In presence of red mud waste cadmium gets adsorbed and cadmium was no more available for the plants to absorb. Red mud waste is a known toxicant and affects algal growth at very low concentrations. The reports of Patnaik et al., (2017, 2018a, b, 2022 and 2023) clearly indicated the deadly toxic nature of red mud waste and leached chemicals of red mud waste. It is a well accepted fact that the toxicity of cadmium is reduced by the red mud waste, as cadmium is adsorbed by red mud waste and did not allow cadmium metal to get absorbed by the plant system. This method of reclamation of cadmium by red mud waste, no doubt decreases the toxicity of cadmium but the toxic chemicals present in the red mud waste can cause detrimental effect on crop plants. Hence neither it was possible to recommend the use of red mud waste to decontaminate the cadmium
polluted environment both aquatic and terrestrial, as the red mud waste will create havoc in the farm lands or crop fields. Carlo et al., (2020) reported long term containment of bauxite residue has been associated with environmental risks due to potential dusting and surface run off. The authors also reported that elevated sodium and aluminium are inhibitory to plant growth. Hence it is not practically possible to use the idea and technology to reduce the toxicity of bauxite residue or cadmium. Li et al., (2019) reported the impact of red mud based- passivator for the remediation of two kinds of cadmium polluted crop field soils and the mechanism of cadmium adsorption. By this process the cadmium pollution reduced by the application of red mud waste is not acceptable in our present piece of work. We are of the opinion that to reduce cadmium toxicity, we should not add red mud which is a complex mixture of many chemicals originating from bauxite mine and time and again declared to be deadly toxic. The red mud waste extract impacted the biochemical machinery of the green gram plants. The depletion in DNA & protein content is a sign of the waste impacting the cells and probably cause death of the cells. Increase in RNA content is a consequence of toxicity and decrease in FAA content might be due to proteolysis caused by the red mud waste extract containing poisonous chemicals. Gautam et al., (2018) identified the indicator species at abandoned red mud dumps in comparison to residential and forest sites, accredited to soil properties. The same author indicated that the dominance of species in those contaminated areas were due to selection strategy in response to change in soil properties and also recommended those select species for plantation in red mud contaminated sites for reclamation. Gautam and Agrawal (2021) reported the remediation of RM waste treated soil by Chrysopogon zizanioides. Li et al., (2019) reported possibility of remediation of cadmium polluted paddy soils and the same authors also explained the mechanism of cadmium adsorption. Fourrier et al., (2021) explained how Raw and Gypsum modified bauxite residues modify and affect seed germination and root development in white mustard, Sinapis alba (L.). Chauhan and Ganguly (2011) standardized the rehabilitation protocol using vegetation cover for bauxite wastes (red mud) in eastern India and indicated the advantages of rehabilitation protocol for reducing the impact of red mud wastes. Misik et al., (2014) reported that red mud is dangerously toxic, caused mutagenicity and also caused DNA damage in humans. The same author also indicated that red mud release in to the environment is not a local problem but a global problem. Li et al., (2018) reported that depletion of cadmium content in rice plant grown in red mud contaminated soil if red mud based passivator was used. They indicated that this can be an effective remediation in cadmium contaminated soils. The same authors also reported the depletion of cadmium content in each part of the rice plant and this looked more effective. Panda et al., (2021) cautioned that restoration of mining over burden disposal area was a challenging effort to minimize environmental impacts and support sustainable agricultural production. From the observed data it is well evident that though no residual accumulation of alumina or non detectable amount of alumina was observed in the seeds of the green gram crop plants but the vegetative biomass contained residual alumina and hence this crop can be recommended for cultivation but the vegetative biomass should not be allowed to be grazed or eaten by grazers or herbivores as alumina might accumulate in the body tissues of grazers, can cause serious illness among them and ultimately human beings residing in the contaminated area.

Acknowledgement

The authors wish to thank the authorities of Berhampur University, Berhampur, Odisha, India for using the laboratory and library facilities. Ms. Patnaik wishes to thank Principal, M M Women’s College, Berhampur, Odisha for permission to conduct the research work.

References


**Declarations:**

**Author Contribution statement**

Dr. A. Leelaveni- Experimental planning and execution of the project, field visit, original draft preparation, supervision, editing. Research work conducted by scholar – Swarnalata Patnaik- red mud waste collection, field study, analysis and related laboratory experimental work. Smt. Patnaik contributed reagents, glassware, field related work, manuscript preparation, calculation and finalization of data. Prof. A.K. Panigrahi- Conceptualization, planning, supervision, field visit, script preparation, reviewing and editing.

**Funding statement:** Authors have not received any fund from any source. All the expenses were borne by Smt. Patnaik, Research Fellow.

**Conflict of interest statement:** The authors declare that they have no conflicts of interest.